acquired SGS at our institution between January 1994 and December 2013 were retrospectively reviewed. Severe SGS was defined as Myer-Cotton grade III (71–99 % obstruction) or grade IV (no detectable lumen) stenosis in this study. Patients with congenital or mixed (congenital stenosis deteriorated by endotracheal intubation) SGS were excluded from this study. Thirty-three patients with severe acquired SGS were identified. The information collected included patient demographics, comorbidities, period and duration of endotracheal intubation, rigid bronchoscopic findings (degree of stenosis according to the Myer-Cotton classification, range of stenosis), details of initial open surgical intervention (single- or double-stage LTR), morbidity and mortality, and the operation-specific decannulation rate (decannulation rate without need for additional surgical treatment). Single-stage LTR was defined as a procedure in which the patient did not have a tracheostomy tube in place at the conclusion of the operation. Patients who underwent double-stage LTR had a tracheostomy tube in place at the conclusion of the procedure. The choice of open surgical intervention methods was left to the surgeons. Single-stage LTR was mainly performed before 2011, and double-stage LTR was mainly performed since 2011. The operation-specific decannulation rate was calculated for the patients in whom decannulation was attempted after initial open surgical intervention, and the patients awaiting decannulation were excluded.

Surgical technique

Single-stage LTR

Costal cartilage with perichondrium was harvested from the sixth or seventh costal arch. After exposing the anterior surface of the larynx with a collar incision, a vertical midline incision was made through the lower third of the thyroid cartilage, the anterior cricoid arch, and the first or second tracheal cartilage ring. A posterior cricoid split without graft was added in patients with severe thickening of the posterior cricoid wall. The harvested costal cartilage was carved to a boat-shaped graft, and lateral wedges of cartilage were trimmed to prevent prolapse into the airway. Endotracheal tube was then placed nasally for postoperative ventilation and stenting. The costal cartilage graft was positioned in the anterior opening and secured in position with interrupted 5–0 absorbable sutures, with the perichondrium facing the airway lumen. At 1–3 weeks after surgery, extubation was performed.

Double-stage LTR

A vertical midline incision of the larynx was made in the same manner as for single-stage LTR. If necessary, the posterior cricoid split was performed. A silicon T-tube (Koken, Inc, Tokyo, Japan) was placed in the subglottic lumen as a stent for the expanded subglottic space. Cartilage expansion, such as in the single-stage procedure, was not performed. After T-tube stenting for at least 6 months, extubation was performed.

Statistical evaluation

Continuous valuables are expressed as medians (range). Mann–Whitney's *U* test was used to compare continuous valuables between the two groups. Fisher's test was used to compare discrete variables between the two groups. All data were analyzed using GraphPad Prism software 6.0 (GraphPad Software, Inc., San Diego, CA, USA). Results showing probability levels <0.05 were considered significant.

Results

Clinical characteristics (Table 1)

Thirty-three patients (16 male, 17 female) who had severe acquired SGS were identified. The median age at diagnosis by rigid bronchoscopy was 1.0 year (range 0.3–6 years). All patients had SGS secondary to endotracheal intubation. Twenty-six patients were intubated in the neonatal period due to respiratory distress syndrome. Seven patients were

Table 1 Clinical data of all patients

Age at diagnosis, median (range)	1.0 year (0.3–6 years)
Sex	Male: 16, female: 17
Period of intubation	
Neonatal period	26
After infancy	7
Duration of intubation, median (range)	4.5 m (0.1–13 m)
Tracheostomy-dependent	33
Severe comorbidities	
No	16
Respiratory	6
Cardiac	5
Neurologic	5
Gastrointestinal	3
Others	2
Stenosis	
Myer-Cotton grade	
III	29
IV	4
Localized at subglottis	9
Extended to glottis or supraglottis	24
Operation-specific decannulation rate	41.7 % (5/12)

intubated after infancy. In the patients intubated after infancy, five patients were due to another operation, one was respiratory tract infection, and another one was a traffic injury. The median duration of intubation was 4.5 months (range 0.1–13 months). All patients had undergone tracheostomy after the diagnosis of acquired SGS. Seventeen patients (52 %) had severe comorbidities.

Rigid bronchoscopic findings (Table 1)

All patients underwent rigid bronchoscopic examination and were diagnosed to have severe SGS. No surgical or endoscopic interventions had been performed before bronchoscopic examination in all patients. According to the Myer-Cotton classification, 29 patients (88 %) had grade III stenosis, and 4 patients (12 %) had grade IV stenosis. Nine patients (27 %) had a stenosis localized at the subglottis (localized SGS; Fig. 1a). Twenty-four patients (73 %) had a stenosis that extended to the glottis or the supraglottis from the subglottis (extended SGS; Fig. 1b).

Localized SGS (Tables 2, 3)

Three patients were intubated in the neonatal period, and six patients were intubated after infancy. The median duration of intubation was 1.0 month (range 0.1–13 months). Seven patients had grade III stenosis, and two patients had grade IV stenosis. Six patients had severe comorbidities. Open surgical intervention was performed in eight patients. The median age at operation was 2.5 years (range 0.75–4 years). Single-stage LTR was performed in four patients, and double-stage LTR was performed in four patients. The postoperative course was uneventful in all patients. In five patients in whom decannulation was attempted after initial surgical intervention, four patients were decannulated (operation-specific decannulation rate: 80.0 %). One patient failed to decannulate due to glottic granulation tissue formation.

Extended SGS (Tables 2, 3)

Twenty-three patients were intubated in the neonatal period, and one patient was intubated after infancy. The median duration of intubation was 5.0 months (range 0.5-12 months). Twenty-two patients had grade III stenosis, and two patients had grade IV stenosis. Eleven patients had severe comorbidities. Open surgical intervention was performed in eight patients. The median age at operation was 2.0 years (range 0.5–9 years). Single-stage LTR was performed in seven patients, and double-stage LTR was performed in one patient. The postoperative course was uneventful in all patients. In seven patients in whom decannulation was attempted after initial surgical intervention, one patient was decannulated (operation-specific decannulation rate: 14.3 %). The causes of failure to decannulate were remaining supraglottic or glottic stenosis in four patients, re-stenosis of the subglottis in two patients, and prolapse of the costal cartilage graft in one patient.

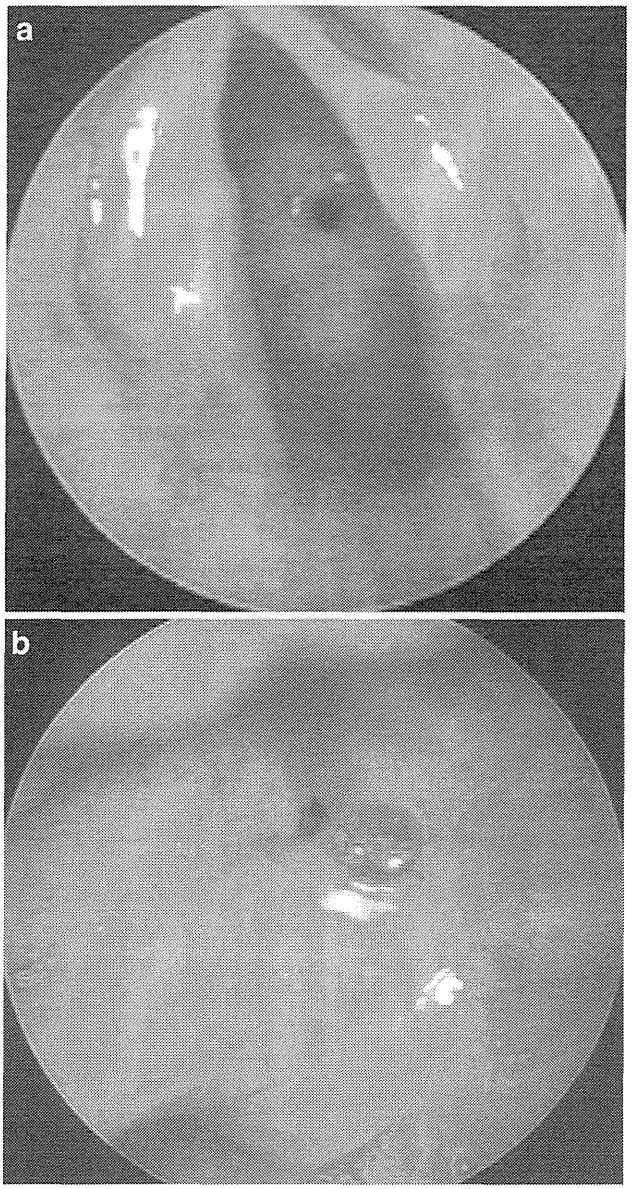

Fig. 1 Rigid bronchoscopic findings of severe acquired subglottic stenosis (SGS). **a** The stenosis is localized at the subglottis (localized SGS). **b** The stenosis extends to the supraglottis from the subglottis (extended SGS)

Discussion

In the last four decades, various techniques of LTR for acquired SGS have been reported. Fearon and Cotton [4] introduced the procedure widening the subglottic lumen

Table 2 Comparison of clinical data (localized SGS versus extended SGS)

	Localized SGS ($n = 9$)	Extended SGS ($n = 24$)	p value
Period of intubation			
Neonatal period	3	23	0.0005
After infancy	6	1	
Duration of intubation, median (range)	1.0 m (0.1–13 m)	5.0 m (0.5–12 m)	0.0024
Myer-Cotton grade			
III	7	22	0.2952
IV	2	2	

Table 3 Comparison of operative data (localized SGS versus extended SGS)

	Localized SGS ($n = 8$)	Extended SGS ($n = 8$)	p value
Age at operation, median (range)	2.5 years (0.75–4 years)	2.0 years (0.5–9 years)	0.6995
Myer-Cotton grade			
III	6	7	1.0000
IV	2	1	
Operation			
Single-stage LTR	4	7	0.2821
Double-stage LTR	4	1	
Operation-specific decannulation rate	80 % (4/5)	14.3 % (1/7)	0.0720

with the interposition of cartilage grafts, and this method has become one of the most common procedures for the treatment of acquired SGS. Single-stage LTR in this study had the same concept as the procedure previously described. Double-stage LTR in this study consisted of anterior cricoid splitting without the interposition of cartilage grafts and T-tube stenting for the widened subglottic space. Anterior cricoid splitting was originally described as a procedure designed to avoid tracheostomy in premature children who had failed extubation due to SGS [5], and Zaima et al. [6] reported this procedure and T-tube stenting as a definitive treatment for severe acquired SGS.

In this study, the overall operation-specific decannulation rate of LTR in patients with severe acquired SGS was 41.7 % (5/12). Experienced centers where PCTR has been performed have reported higher operation-specific decannulation rates (71–76 %) in patients with severe SGS [2, 3]. Although PCTR is regarded as the preferred option for severe SGS over LTR [7], it is not clear whether LTR is suitable for all types of severe acquired SGS or not. The Myer-Cotton classification [8], a grading system according to the degree of subglottic luminal obstruction, is widely used for evaluation of SGS. This grading system is simple and useful for assessing clinical outcomes. On the other hand, various forms and ranges of stenosis are present in the same Myer-Cotton grade, and this grading system does not always represent the true conditions of SGS. Monnier et al. [9] pointed out the limitation of the Myer-Cotton classification, and they proposed a new classification based on the degree of the stenosis, associated comorbidities, and/or glottis involvement. In this study, the range of stenosis was assessed in addition to the degree of luminal obstruction based on bronchoscopic findings. Consequently, acquired SGS is classified as follows based on bronchoscopic findings: (1) stenosis localized at the subglottis (localized SGS), and (2) stenosis extending to the glottis or supraglottis from the subglottis (extended SGS).

Many patients with localized SGS were intubated after infancy, and the duration of intubation was significantly shorter in patients with localized SGS than in those with extended SGS. These facts suggest that the range of stenosis is affected by the size of the subglottic lumen and the duration of intubation, and the subglottis, the narrowest part of the pediatric airway, is selectively damaged in localized SGS. Hartley and Cotton [7] suggested that PCTR was the preferred surgical option for localized SGS. Because of a clear margin between the stenosis and the vocal cords, complete resection of the stenotic segment and safe thyrotracheal anastomosis are possible. They argued that at least a 3-mm margin of tissue between the stenosis and the vocal cords is recommended for PCTR. On the other hand, the operation-specific decannulation rate of LTR in patients with localized SGS was 80.0 % in the present study. The present results indicate that LTR is a feasible surgical option for severe localized SGS taking into consideration technical simplicity and minimal invasiveness.

Most patients with extended SGS required prolonged intubation from newborns. We consider that a wide range of the larynx through the glottis or supraglottis to the subglottis could be damaged in the extended SGS group due to the small size of the larynx and the long duration of intubation. The present study showed a low operation-

specific decannulation rate in patients with extended SGS (14.3 %). Major causes of failure to decannulate were re-stenosis of the subglottis and remaining supraglottic or glottic stenosis. LTR is a procedure aimed at enlarging the subglottic lumen by vertical incision of the cricoid ring and stabilizing the expanded lumen with a costal cartilage graft or T-tube stenting. Since much cicatricial tissue remains after LTR in extended SGS, re-stenosis of the subglottis is a possible postoperative complication [10]. These facts suggest that PCTR, a procedure with resection of almost all cicatricial tissue, is an effective procedure for the treatment of extended SGS. Furthermore, additional procedures for releasing the stenosis of the glottis or supraglottis, such as laryngoscopic scar excision or separation of fused vocal cords, are necessary for decannulation. George et al. [11] reported a high operation-specific decannulation rate of 61 % after PCTR with an additional procedure for SGS with glottic involvement.

This study has several important limitations. First, the study population was small. Second, although the concept of the surgical intervention procedures widening the subglottic lumen by vertical incision of the cricoid ring was the same, two procedures (single- or double-stage LTR) were performed in this study. Further studies including a large number of participants with the same procedure are needed to clarify which procedure is feasible for each type of severe acquired SGS.

In conclusion, the range of stenosis was affected by the period and duration of endotracheal intubation, and surgical outcomes of LTR tended to differ between localized SGS and extended SGS. LTR can be an effective procedure for the treatment of severe localized SGS. Careful bronchoscopic evaluation about the range of stenosis in addition to the degree of stenosis is necessary to predict the outcome.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

References

1. Cotton RT (1985) Prevention and management of laryngeal stenosis in infants and children. J Pediatr Surg 20:845–851
2. White DR, Cotton RT, Bean JA, Rutter MJ (2005) Pediatric cricotracheal resection: surgical outcomes and risk factor analysis. Arch Otolaryngol Head Neck Surg 131:896–899
3. George M, Ikonomidis C, Jaquet Y, Monnier P (2009) Partial cricotracheal resection in children: potential pitfalls and avoidance of complications. Otolaryngol Head Neck Surg 141:225–231. doi:10.1016/j.otohns.2009.04.019
4. Fearon B, Cotton R (1972) Surgical correction of subglottic stenosis of the larynx. Preliminary report of an experimental surgical technique. Ann Otol Rhinol Laryngol 81:508–513
5. Cotton RT, Seid AB (1980) Management of the extubation problem in the premature child. Anterior cricoid split as an alternative to tracheotomy. Ann Otol Rhinol Laryngol 89:508–511
6. Zaima A, Bitoh Y, Morita K, Tsugawa J, Ishii T, Satoh S, Nishijima E (2010) Long-term T-tube stenting as definitive treatment of severe acquired subglottic stenosis in children. J Pediatr Surg 45:996–999
7. Hartley BE, Cotton RT (2000) Paediatric airway stenosis: laryngotracheal reconstruction or cricotracheal resection? Clin Otolaryngol Allied Sci 25:342–349
8. Myer CM 3rd, O'Connor DM, Cotton RT (1994) Proposed grading system for subglottic stenosis based on endotracheal tube sizes. Ann Otol Rhinol Laryngol 103:319–323
9. Monnier P, Ikonomidis C, Jaquet Y, George M (2009) Proposal of a new classification for optimising outcome assessment following partial cricotracheal resections in severe pediatric subglottic stenosis. Int J Pediatr Otorhinolaryngol 73:1217–1221. doi:10.1016/j.ijporl.2009.05.008
10. Yamamoto K, Monnier P, Holtz F, Jaquet Y (2014) Laryngotracheal reconstruction for pediatric glotto-subglottic stenosis. Int J Pediatr Otorhinolaryngol 78:1476–1479. doi:10.1016/j.ijporl.2014.06.012
11. George M, Jaquet Y, Ikonomidis C, Monnier P (2010) Management of severe pediatric subglottic stenosis with glottic involvement. J Thorac Cardiovasc Surg 139:411–417. doi:10.1016/j.jtcvs.2009.05.010

Everolimus for Primary Intestinal Lymphangiectasia With Protein-Losing Enteropathy

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abstract

Primary intestinal lymphangiectasia (PIL), also known as Waldmann's disease, is an exudative enteropathy resulting from morphologic abnormalities in the intestinal lymphatics. In this article, we describe a 12-year-old boy with PIL that led to protein-losing enteropathy characterized by diarrhea, hypoalbuminemia associated with edema (serum albumin level: 1.0 g/dL), and hypogammaglobulinemia (serum IgG level: 144 mg/dL). Severe hypoalbuminemia, electrolyte abnormalities, and tetany persisted despite a low-fat diet and propranolol. Everolimus (1.6 mg/m^2/day) was added to his treatment as an antiangiogenic agent. With everolimus treatment, the patient's diarrhea resolved and replacement therapy for hypoproteinemia was less frequent. Hematologic and scintigraphy findings also improved (serum albumin level: 2.5 g/dL). There were no adverse reactions during the 12-month follow-up. To the best of our knowledge, this is the first report of everolimus use in a patient with PIL.

Lymphatic anomalies include cystic lymphatic malformation, generalized lymphatic anomaly, Gorham–Stout disease, lymphangiectasia, and central conducting lymphatic disorders.[1,2] Lymphangiectasia occurs as a primary developmental lymphatic disorder with or without elevated systemic venous pressure due to lymphatic obstruction, and the condition must be distinguished from other lymphatic disorders. Depending on the site of the anomaly, lymphangiectasia may manifest as chylothorax, pulmonary lymphangiectasia, chylous ascites, protein-losing enteropathy (PLE), cutaneous vesicles, or superficial chylous leaks.[3]

Primary intestinal lymphangiectasia (PIL), also known as Waldmann's disease, is a rare exudative enteropathy characterized by morphologic abnormalities of the intestinal lymphatics without proliferation.[4] Common symptoms of PIL are persistent diarrhea, peripheral edema, steatorrhea, lymphocytopenia, hypogammaglobulinemia, and hypoproteinemia. Treatment is generally symptomatic and may include a low-fat diet associated with medium-chain triglycerides, periodic intravenous albumin infusion, and corticosteroid administration.[5] In a more severe case, octreotide, a synthetic analog of the naturally occurring hormone somatostatin (a potent inhibitor of the release of growth hormone, serotonin, gastrin, glucagon, and insulin), was successful.[6] However, no curative therapy is currently available for PIL. A US Federal Drug Administration–funded prospective study of sirolimus, a mammalian target of rapamycin (mTOR) inhibitor, in patients with complicated vascular anomalies has been underway (ClinicalTrials.

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Dr Ozeki contributed to the study conception and design, drafted the initial manuscript, and approved the final manuscript as submitted; Drs Hori, Kanda, and Kawamoto provided patient care, performed clinical data analyses, reviewed and revised the manuscript, and approved the final manuscript as submitted; Dr Ibuka performed gastroenterological examinations, endoscopy, and clinical data analyses; reviewed and revised the manuscript; and approved the final manuscript as submitted; Dr Miyazaki carried out pathological examinations and analyses, reviewed and revised the manuscript, and approved the final manuscript as submitted; Dr Fukao contributed to the study concept and design and critically reviewed the manuscript; and all authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

DOI: 10.1542/peds.2015-2562

Accepted for publication Nov 19, 2015

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PEDIATRICS (ISSN Numbers: Print, 0031-4005; Online, 1098-4275).

FINANCIAL DISCLOSURE: The authors have indicated they have no financial relationships relevant to this article to disclose.

FUNDING: This study was supported in part by a grant-in-aid 25461587 for Scientific Research from

To cite: Ozeki M, Hori T, Kanda K, et al. Everolimus for Primary Intestinal Lymphangiectasia With Protein-Losing Enteropathy. *Pediatrics.* 2016;137(3):e20152562

gov; identifier NCT00975819) since 2009.[7] The successful use of mTOR inhibitors in the treatment of a small collection of lymphatic anomalies has recently been reported.[8] Therefore, we considered that an mTOR inhibitor might be a promising treatment for PIL.

In this article, we present a case of a patient with PIL and severe PLE who benefitted from systemic therapy with everolimus, an mTOR inhibitor. To our knowledge, this is the first report of everolimus therapy in the treatment of PIL. The findings provide insight into the biology underlying lymphatic anomalies and support further study of mTOR inhibitors as a treatment for PIL.

CASE REPORT

A 12-year-old Japanese boy presented to a municipal hospital with a 3-month history of progressive diarrhea, abdominal pain, lower limb edema, tetany, and weakness. He had no relevant family history or medical history. The patient was provisionally diagnosed with PIL and secondary PLE and treated with a low-fat diet associated with medium-chain triglycerides, antiflatulent medication (*Bifidobacterium*), antidiarrheal medication (loperamide hydrochloride and natural aluminum silicate), furosemide, and sodium cromoglycate. However, he experienced progressively worsening refractory nonbloody diarrhea, edema, and hypoalbuminemia (1.0 g/dL) (lower limit of normal for age: 3.8 g/dL), for which he received periodic albumin infusions (4 infusions of 12.5 g) and immunoglobulin (2 infusions of 5.0 g) for infection prophylaxis. After 4 months, the patient was transferred to Gifu University Hospital to establish a definitive diagnosis and undergo additional therapy.

Physical examination on admission revealed an acutely ill patient with a puffy face and generalized edema. Laboratory findings revealed decreased levels of serum albumin (1.4 g/dL), total protein (2.8 g/dL), magnesium (0.9 mg/dL), and corrected calcium (8.1 mg/dL). Hypogammaglobulinemia was noted; at their nadirs, the IgM level was 60 mg/dL (normal: 45–300 mg/dL), IgA was 71 mg/dL (normal: 95–460 mg/dL), and IgG was 226 mg/dL (normal: 890–1850 mg/dL). The urinalysis results were within normal limits. The patient had a high fecal α-1-antitrypsin clearance rate (494 mL/day; normal: <20 mL/day), indicating enteric loss of plasma proteins. Gastrointestinal endoscopy showed white villi and chyle leakage in the mucosa of the distal duodenum and ascending colon to the transverse colon (Fig 1A). Histologic examination of biopsy specimens from the duodenum and ileocecum showed diffusely dilated mucosal and submucosal lymphatic channels along the villi (Fig 1B). Staining for the lymphatic marker D2-40 was positive in the luminal cells (Fig 1C). Additionally, we found elevated nuclear and cytoplasmic immunohistochemical expression of mTOR in the lymphatic endothelial cells (Fig 1D). ^{99m}Tc human serum albumin (^{99m}Tc-HSA) scintigraphy showed albumin leakage from the ascending colon to the transverse colon (Fig 2A). Scintigraphic examination showed no leakage in the stomach or small bowel. These results allowed for a definitive diagnosis of PIL and secondary PLE. Management of the patient's hypoalbuminemia with a low-fat diet and infusions of albumin (12.5 g) with furosemide was unsuccessful. Because of this treatment failure, the patient was treated with oral propranolol (3 mg/kg per day divided every 8 hours). After 4 weeks, the patient's symptoms persisted, and we decided to initiate treatment with an mTOR inhibitor. Because sirolimus was not available in Japan at that time, we chose another mTOR inhibitor, everolimus, which is approved for immunosuppressive therapy. The treatment was approved by the review board at our hospital, and written informed consent was obtained from the patient's parents. Everolimus was started at 2 mg (1.6 mg/m^2 per day). Dose adjustments were made to maintain the desired drug trough level of 5 to 15 ng/mL according to the literature.[8] At the start of everolimus treatment, the serum albumin level was 1.6 g/dL and the IgG level was 217 mg/dL. After 4 weeks, the patient's diarrhea had almost resolved and his serum albumin level was gradually increasing. Six months after initiation of everolimus, ^{99m}Tc-HSA scintigraphy showed no leakage of albumin from the gastrointestinal tract (Fig 2B). Fecal α-1-antitrypsin clearance was markedly decreased (177 mL/day). However, the objective endoscopic findings in the duodenum were unchanged. The patient's severe hypoalbuminemia gradually improved and the administration of immunoglobulin and albumin became less frequent, although the serum albumin level did not normalize (Fig 3). The patient's treatment continued without any adverse effects during 12 months of follow-up.

DISCUSSION

PIL is a chronic debilitating disorder requiring strict long-term dietary control based on a low-fat regimen and supplementary medium-chain triglycerides. Surgical small-bowel resection is useful in the rare cases of segmental and localized PIL.[9] PIL negatively affects quality of life and can be life-threatening when malignant complications or serous effusions occur. There is no consensus on the treatment of this condition. Treatment is generally symptomatic and may include nutritional therapy and replacement therapy. A few patients effectively treated with propranolol

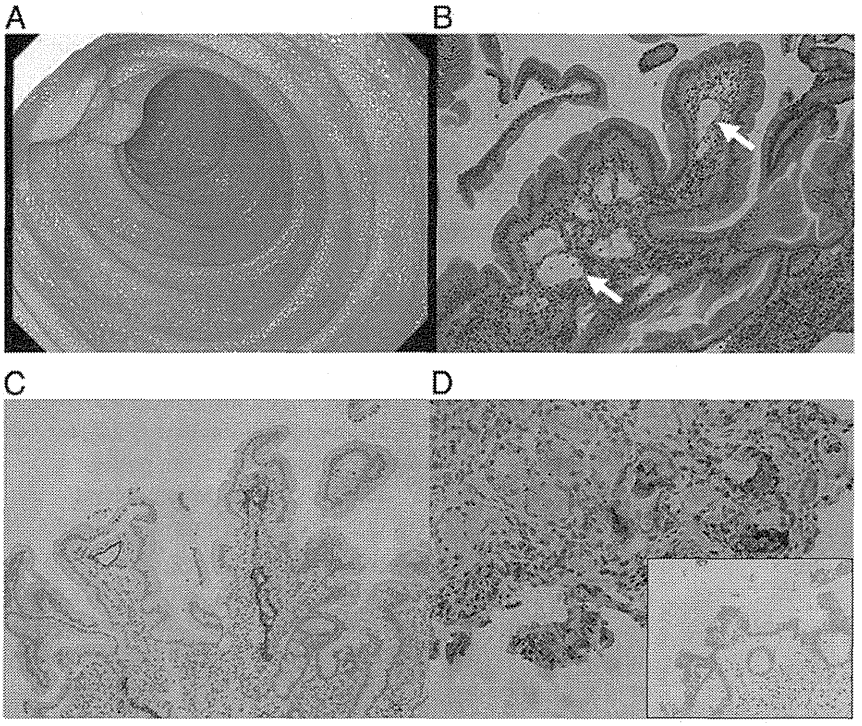

FIGURE 1
A, Enteroscopy revealed white villi and chyle leakage in the intestinal mucosa. B, Histopathology of intestinal tissue before treatment showed marked dilatation of lymphatic ducts along a villus (arrow) and involving the mucosa (hematoxylin and eosin stain, ×400). C, D2-40 immunostaining was positive in the luminal endothelial cells. D, Elevated nuclear and cytoplasmic immunohistochemical expression of mTOR was found in the lymphatic endothelial cells. Anti-mTOR immunohistochemistry manifested brown positive signals. The reference image in the bottom-right corner revealed normal intestinal mucosa.

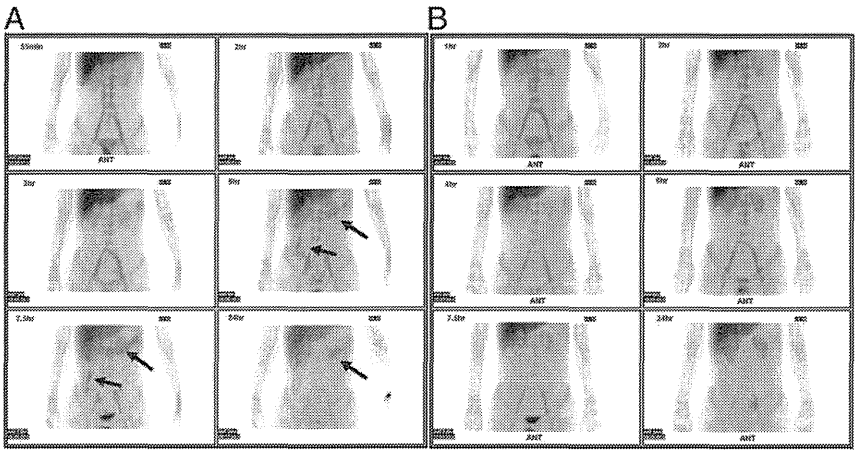

FIGURE 2
A, ^{99m}Tc-HSA scintigraphy before treatment showed leakage of albumin from the ascending colon to the transverse colon (arrows). B, ^{99m}Tc-HSA scintigraphy after 6 months of everolimus administration showed no leakage of albumin from the gastrointestinal tract.

for lymphatic malformation and generalized lymphatic anomalies were recently described.[10,11] However, propranolol did not show a therapeutic effect on the condition of our patient. We have presented a case of PIL with PLE treated with everolimus. This agent resulted in marked improvement in the patient's symptoms and examination findings.

The successful use of mTOR inhibitors in the treatment of a small collection of vascular anomalies has recently been reported.[7] mTOR is a serine/threonine kinase regulated by phosphoinositide-3-kinase. It acts as a master switch for numerous cellular processes, including cellular catabolism and anabolism, cell motility, angiogenesis, and cell growth.[12] In our patient, significant expression of mTOR was found in the affected tissues. Based on this finding, we propose that the enhanced mTOR expression seen within abnormal tissues affected by lymphangiectasia may explain the efficacy and mechanism of mTOR inhibitors in treating PIL lesions and may predict the efficacy of mTOR inhibitor therapy.

The etiology of PIL currently remains unknown. Several genes, including vascular endothelial growth factor receptor 3, prospero-related homeobox-transcriptional factor, forkhead transcriptional factor, and SRY (sex determing region Y)-Box 18 (SOX18), are involved in the development of the lymphatic system. Vascular endothelial growth factor is a key regulator in lymphangiogenesis and angiogenesis, and it acts as both a potential upstream stimulator of the mTOR signaling pathway and a downstream effector. Hokari et al.[13] reported inconsistently changes in the expression of lymphangiogenesis regulatory molecules in the duodenal mucosa of patients with PIL. The lymphangiogenesis pathway is assumed to be involved in PIL, in which ligand binding-induced signaling through vascular endothelial growth factor receptor 3 on the surface of the lymphatic endothelium results in activation of the phosphoinositide-3-kinase /Akt/ mTOR pathway. Therefore, mTOR inhibitors are predicted to be effective agents for PIL.

The mechanisms of enteric protein loss in intestinal lymphangiectasia

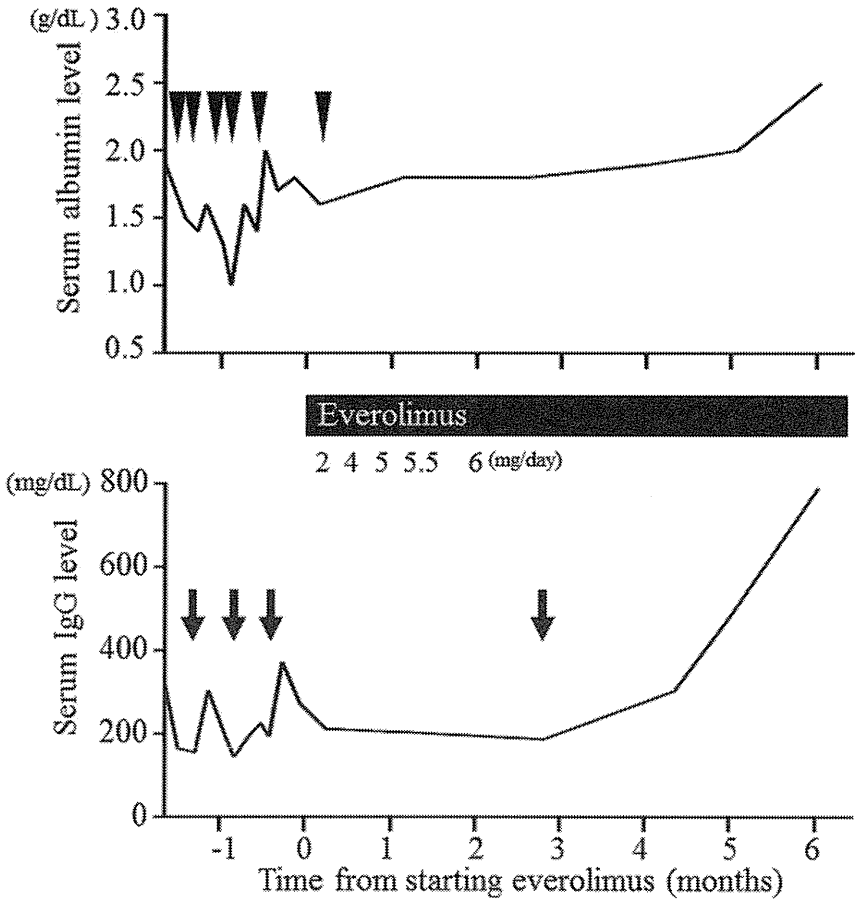

FIGURE 3
Serum albumin and IgG levels in relation to everolimus therapy in the patient. Inverted triangle indicates intravenous albumin infusion (12.5 g). Downward-pointing arrow indicates intravenous immunoglobulin infusion (5.0 g). Horizontal axis indicates time from the start of everolimus therapy.

are not well understood, although increased pressure in the lymph channels has been suggested as a possible cause.[14] Lymphatic hypoplasia results in obstruction of lymph flow, which leads to increased pressure within the lymphatics. This, in turn, causes dilation of the lymphatic channels in the intestine and leads to rupture of the channels with resultant loss of lymph into the bowel lumen.[15] Lymphangiography is a valuable tool for the detection of lymphatic leakage. Recently published reports indicate that lymphangiography plays a therapeutic role in patients with lymphatic leakage.[16] In our case, although lymphangiography was not performed, scintigraphy and α-1-antitrypsin clearance showed lymphatic leakage and obvious improvement with treatment. However, there were no changes in the enteroscopic findings after treatment. Everolimus might have effects on the function of the lymphatic canals, but no apparent effects on endoscopic findings.

Everolimus has been approved for use in Japan as an immunosuppressant for the prevention of cardiac and renal allograft rejection.[17] Practical experience has revealed that everolimus has some benefits over sirolimus. Although both substances are fairly similar chemically, the serum half-life of everolimus is shorter than that of sirolimus (28 vs 62 hours, respectively). Everolimus is more controllable because it reaches steady state earlier than does sirolimus (4 vs 6 days, respectively).[18] Additionally, everolimus is reportedly associated with a lower incidence of hyperlipidemia.[19] Although serious side effects are rare, it is necessary to monitor for their occurrence with any mTOR inhibitor therapy.

CONCLUSIONS

To the best of our knowledge, this is the first report of everolimus use in a patient with PIL. The clinical response to mTOR inhibitors such as sirolimus and everolimus may be related to inhibition of lymphatic endothelial cell growth and improvement in lymphatic canal function. Prospective studies are needed to determine the best systemic therapies for PIL with PLE and the optimal duration of treatment.

ACKNOWLEDGMENTS

We thank Dr Chiemi Saigo of Gifu University, Dr Hideki Matsumoto of Gifu Prefectural General Medical Center, and Drs Emi Kadoi and Kunihiro Shinoda of Gifu Municipal Hospital for their helpful comments. We also thank MT Yasuo Katagiri and MT Atsushi Nakagawa of Gifu University for technical assistance and the Department of Pediatrics at Gifu University for their contributions.

ABBREVIATIONS

mTOR: mammalian target of rapamycin
PIL: primary intestinal lymphangiectasia
PLE: protein-losing enteropathy
^{99m}Tc-has: ^{99m}Tc human serum albumin

the Ministry of Education, Culture, Sports, Science and Technology of Japan; Health and Labour Science Research Grants for Research on Intractable Diseases from the Ministry of Health, Labour and Welfare of Japan received by M.O.; and the Practical Research Project for Rare/Intractable Diseases from Japan's Agency for Medical Research and Development, AMED (15Aek0109057h0102).

POTENTIAL CONFLICT OF INTEREST: The authors have indicated they have no potential conflicts of interest to disclose.

REFERENCES

1. Trenor CC III, Chaudry G. Complex lymphatic anomalies. *Semin Pediatr Surg.* 2014;23(4):186–190
2. International Society for the Study of Vascular Anomalies. ISSVA classification for vascular anomalies. Available at: www.issva.org. Accessed May 2, 2015
3. Malone LJ, Fenton LZ, Weinman JP, Anagnost MR, Browne LP. Pediatric lymphangiectasia: an imaging spectrum. *Pediatr Radiol.* 2015;45(4):562–569
4. Vignes S, Bellanger J. Primary intestinal lymphangiectasia (Waldmann's disease). *Orphanet J Rare Dis.* 2008;3(5). Available at: www.ojrd.com/content/3/1/5
5. Binder HJ. Disorders of absorption. In: Brawnwald E, Fauci AS, Kasper DL, Hauser SL, Longo DL, Jameson JL, eds. *Harrison's principles of internal medicine,* 15th ed. New York: McGraw Hill; 2001:1665–1679
6. Kuroiwa G, Takayama T, Sato Y, et al. Primary intestinal lymphangiectasia successfully treated with octreotide. *J Gastroenterol.* 2001;36(2):129–132
7. Hammill AM, Wentzel M, Gupta A, et al. Sirolimus for the treatment of complicated vascular anomalies in children. *Pediatr Blood Cancer.* 2011;57(6):1018–1024
8. Lackner H, Karastaneva A, Schwinger W, et al. Sirolimus for the treatment of children with various complicated vascular anomalies. *Eur J Pediatr.* 2015;174(12):1579–1584
9. Persić M, Browse NL, Prpić I. Intestinal lymphangiectasia and protein losing enteropathy responding to small bowel restriction [letter]. *Arch Dis Child.* 1998;78(2):194
10. Ozeki M, Kanda K, Kawamoto N, et al. Propranolol as an alternative treatment option for pediatric lymphatic malformation. *Tohoku J Exp Med.* 2013;229(1):61–66
11. Ozeki M, Fukao T, Kondo N. Propranolol for intractable diffuse lymphangiomatosis. *N Engl J Med.* 2011;364(14):1380–1382
12. Vignot S, Faivre S, Aguirre D, Raymond E. mTOR-targeted therapy of cancer with rapamycin derivatives. *Ann Oncol.* 2005;16(4):525–537
13. Hokari R, Kitagawa N, Watanabe C, et al. Changes in regulatory molecules for lymphangiogenesis in intestinal lymphangiectasia with enteric protein loss. *J Gastroenterol Hepatol.* 2008;23(7 Pt 2):e88–e95
14. Mistilis SP, Skyring AP, Stephen DD. Intestinal lymphangiectasia. Mechanism of enteric loss of plasma-protein and fat. *Lancet.* 1965;1(7376):77–79
15. Toskes P. Gastrointestinal diseases: malabsorption. In: Wyngaarden J, Smith L, eds. *Cecil Textbook of Medicine,* 18th ed. Philadelphia: WB Saunders; 1988:732–745
16. Matsumoto T, Yamagami T, Kato T, et al. The effectiveness of lymphangiography as a treatment method for various chyle leakages. *Br J Radiol.* 2009;82(976):286–290
17. Chapman TM, Perry CM. Everolimus. *Drugs.* 2004;64(8):861–872, discussion 873–874
18. Pascual J, Boletis IN, Campistol JM. Everolimus (Certican) in renal transplantation: A review of clinical trial data, current usage and future directions. *Transplant Rev.* 2006;20(1):1–18
19. Tenderich G, Fuchs U, Zittermann A, Muckelbauer R, Berthold HK, Koerfer R. Comparison of sirolimus and everolimus in their effects on blood lipid profiles and haematological parameters in heart transplant recipients. *Clin Transplant.* 2007;21(4):536–543

BRIEF REPORT
Gorham–Stout Disease of the Skull Base With Hearing Loss: Dramatic Recovery and Antiangiogenic Therapy

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Gorham–Stout disease (GSD) is a rare disorder of unknown etiology. We present a 6-year-old male with GSD involving the skull base who presented with recurrent cerebrospinal fluid (CSF) rhinorrhea, severe hearing loss, and facial palsy secondary to cerebellar herniation into the internal auditory canal. After 2 months of treatment with pegylated interferon (IFN) α-2b (50 μg/week), his hearing recovered dramatically. Two years later, new bone formation appeared radiologically and IFN was switched to sirolimus. One year after the switch, CSF rhinorrhea disappeared. Antiangiogenic therapy might inhibit proliferation of vascular endothelial cells in osteolytic lesions and lead to new bone formation. Pediatr Blood Cancer © 2015 Wiley Periodicals, Inc.

Key words: cerebrospinal fluid leakage; interferon; lymphatic malformation; mammalian target of rapamycin; sirolimus

INTRODUCTION

Gorham–Stout disease (GSD), also known as massive osteolysis, is a rare bone disorder of unknown etiology originally described in the 1950s.[1] Aggressive lymphatic and vascular proliferation of unknown origin is believed to be the cause of local bone destruction. Michael et al. summarized the clinical information from 185 previously published cases of GSD and found skull base involvement in only nine cases.[2] GSD has a risk of fatal complications. Spinal cord damage and paraplegia resulting from vertebral osteolysis has been reported, as has cerebrospinal fluid (CSF) leakage resulting from osteolysis of the skull.[3,4]

Optimal treatment for GSD has not yet been established. Current therapeutic modalities include surgery, interferon (IFN), and radiotherapy.[2] However, these therapies have many side effects, and treatment strategy remains controversial. Since 2009, a FDA-funded prospective trial of sirolimus, a mammalian target of rapamycin (mTOR) inhibitor, has been underway for treatment of complicated vascular anomalies (ClinicalTrials.gov NCT00975819).[5] mTOR is a serine/threonine kinase regulated by phosphoinositide 3-kinase. It acts as a master switch for numerous cellular processes, including cellular catabolism and anabolism, cell motility, angiogenesis, and cell growth.[6] Recently, the successful use of mTOR inhibitors in treating a small number of lymphatic anomalies has been reported.[7] Therefore, we hypothesized that sirolimus might be a promising treatment for GSD.

Here, we present the case of a child with GSD involving the skull base who presented with CSF leakage, severe hearing loss, and facial palsy secondary to herniation of cerebellar tissue into the internal auditory canal (IAC). Systemic medication could improve symptoms and result in new bone formation. We also discuss this rare condition and the mechanism of recovery.

CASE REPORT

A 3-year-old male presented with a history of repeated CSF rhinorrhea and bacterial meningitis, with left ear deafness as a complication of bacterial meningitis. He was diagnosed with basal meningoencephalocele; surgical repair of the CSF fistula at the anterior skull base was performed at another hospital when the patient was 4 years old. However, he experienced refractory CSF rhinorrhea and bacterial meningitis. When the patient was 6 years old, a skull base biopsy showed resorption of typical bone structures, which had been replaced by thin-walled endothelium-lined capillaries of vascular or lymphatic origin (Fig. 1A). D2-40 immunostaining delineated the endothelium of the lymphatic channels and a diagnosis of GSD was made (Fig. 1B). Six months after biopsy, the patient presented with acute right-sided hearing loss and mild peripheral facial palsy. Because these symptoms persisted for 3 months, he was transferred to our hospital for additional therapy.

The patient had mild right facial nerve palsy (House–Brackmann Facial Nerve Grading System, Grade II, mild dysfunction) and repeated CSF rhinorrhea several times daily. Puretone audiogram demonstrated severe sensorineural hearing loss

Abbreviations: GSD, Gorham–Stout disease; CSF, cerebrospinal fluid; IFN, interferon; FDA, Food and Drug Administration; mTOR, mammalian target of rapamycin; IAC, internal auditory canal; CT, computed tomography; MRI, magnetic resonance imaging; VEGF, vascular endothelial growth factor

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Grant sponsor: Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan; Grant number: 25461587; Grant sponsor: Health and Labour Science Research Grant for Research on Intractable Diseases from the Ministry of Health, Labour and Welfare of Japan (to M.O.); Grant sponsor: Practical Research Project for Rare/Intractable Diseases from Japan's Agency for Medical Research and Development, AMED (15Aek0109057h0102).

Conflict of interest: Nothing to declare.

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Received 13 October 2015; Accepted 4 December 2015

DOI 10.1002/pbc.25886
Published online in Wiley Online Library (wileyonlinelibrary.com).

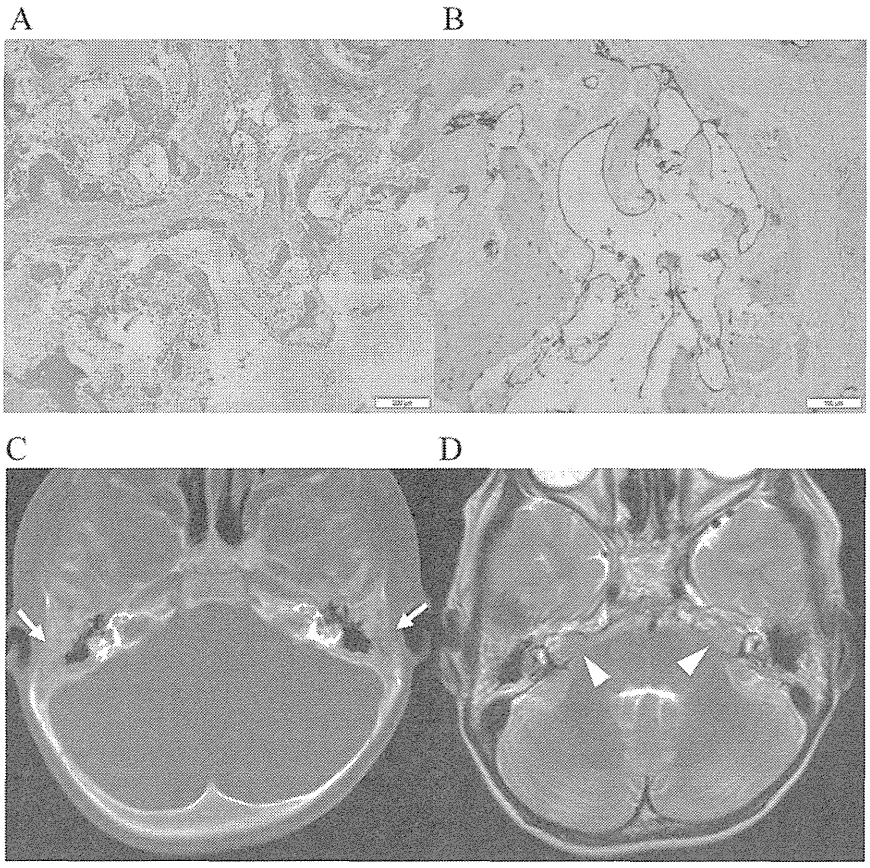

Fig. 1. Pathological examination of the affected lesion and radiological examination of the patient before treatment. (**A**) Skull base biopsy showing that typical bone structures were resorbed and replaced by thin-walled endothelium-lined capillaries of vascular or lymphatic origin (bar 200 μm, hematoxylin and eosin). (**B**) Immunopathological examination positive for lymphatic marker D2-40 (bar 100 μm). (**C**) Axial noncontrast CT of temporal bones revealed diffuse osteolysis (arrow) and severe expansion of the IAC. (**D**) Axial noncontrast T2-weighted MRI revealed herniation of the cerebellar tissue into the IAC (arrowhead).

of the right ear. Auditory-evoked brain response testing of the right ear showed no waveforms at 130 dB sound pressure level. Computed tomography (CT) of the head showed multiple lytic lesions in the temporal bones and expansion of the bilateral IACs (Fig. 1C). Magnetic resonance imaging (MRI) of the skull base demonstrated herniation of cerebellar tissue into the bilateral IACs (Fig. 1D). Radioisotope cisternography with In-111 showed CSF leakage. Because the patient's condition was severe and progressive, we decided to treat with pegylated IFN α-2b. The treatment was approved by the review board at our hospital and written informed consent was obtained from the patient's parents. We started pegylated IFN α-2b (50 μg, 1.5 μg/kg) once weekly, according to a reported protocol.[8] Two months later, the patient's hearing in the right ear recovered dramatically. His audiogram also showed marked improvement. Auditory-evoked brain response showed restoration of I–V waves. Facial nerve function improved to Grade I. However, MRI and CT showed no marked changes in the temporal bones. Six months after initiation of IFN therapy, CT revealed slight new bone formation in the bilateral temporal bones. Two years after IFN therapy was initiated, bone formation was clearly identified in the bilateral temporal bones (Fig. 2A). MRI showed improvement of cerebellar herniation (Fig. 2B). Because the patient had side effects with IFN treatment (high fever, fatigue, and mild depression), the patient and his family wanted to taper the medication. Additionally, the patient continued to experience recurrent CSF rhinorrhea. A clinical study of sirolimus for the treatment of complex vascular anomalies has been underway at our hospital since 2014. We decided to switch this patient's therapy from IFN to sirolimus to minimize side effects and to treat his recurrent CSF rhinorrhea. Written informed consent for this treatment was obtained from the patient's parents.

Sirolimus was added at 1.6 mg/m^2 once daily. Dose adjustments were made to maintain the desired 24-h trough level at 10–15 ng/ml. After 6 months of sirolimus treatment, the frequency of CSF rhinorrhea decreased to once monthly and IFN therapy was discontinued. One year later, the patient's CSF rhinorrhea disappeared completely. Radioisotope cisternography with In-111 showed little leakage of CSF. At 3-year follow-up, the patient had been taking sirolimus for over 1 year without any adverse effects and had good facial nerve function and hearing.

DISCUSSION

Our patient with GSD not only had CSF rhinorrhea, but also sudden severe hearing loss and facial palsy caused by herniation

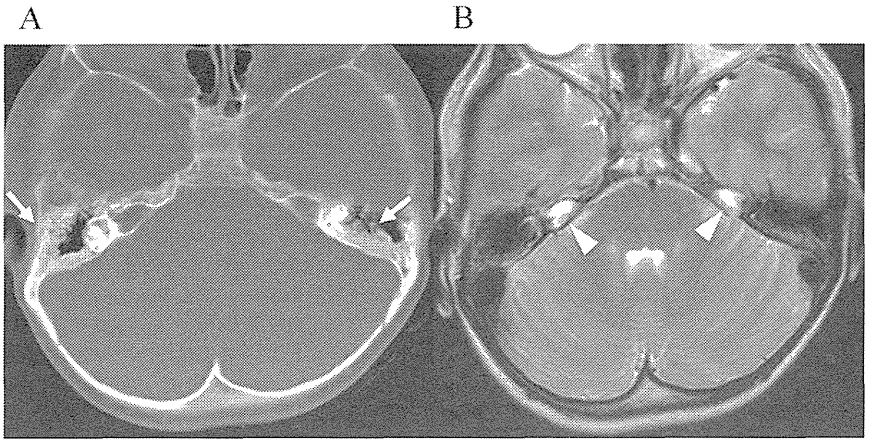

Fig. 2. Radiological examination of the patient 2 years after initiation of interferon therapy and prior to sirolimus treatment. (**A**) Axial noncontrast CT revealed an obvious increase in bone density of the mastoid air cells (arrow). (**B**) Axial noncontrast T2-weighted MRI revealed improvement of cerebellar hernia (arrowhead).

of cerebellar tissue into the right IAC, as a result of an osteolytic lesion of the skull base causing bone fragility. His hearing disturbance and CSF rhinorrhea resolved completely, and new bone was formed in the osteolytic lesion.

The pathogenesis of GSD is poorly understood. There are only three reported cases of hearing loss secondary to GSD.[9–11] In one of these patients, hearing loss was caused by an osteolytic lesion directly infiltrating the cochlear duct. However, the causes of hearing loss in the other two patients were unclear. Hearing was not restored in these patients. We believe that our rare experience provides important insights into the pathogenesis of GSD.

In our patient, cochlear nerve dysfunction and facial nerve palsy developed and resolved concurrently. Patients with cerebellopontine angle meningioma with IAC involvement present with hearing loss and abnormal facial motor function, clinical manifestations caused by compression neuropathy rather than parenchymal involvement.[12,13] It is hypothesized that compression neuropathy results in biochemical changes within the axons and Schwann sheaths of the cerebral nerve, and that segmental demyelination may be implicated.[14] Our patient also had herniation of cerebellar tissue into the IAC, and there was marked reduction of cerebellar herniation when hearing loss recovered. We believe that cerebellar herniation might have caused compression neuropathy of the cochlear and facial nerves. Improvement in cerebellar herniation thus could result in concurrent resolution of hearing loss and facial palsy.

Antiosteoclastic medications (bisphosphonates) are often used to treat GSD.[2] We did not administer bisphosphonates in the present case because the biopsy specimen did not show the presence of osteoclasts or activation of these cells. Instead, our patient received antiangiogenic therapy with pegylated IFN α-2b and sirolimus. Recent studies have suggested that vascular proliferation might be related to the pathogenesis of bone resolution in GSD patients.[15,16] IFN might downregulate expression of vascular endothelial growth factor (VEGF).[17] The mTOR inhibitor sirolimus targets protein synthesis downstream of the Akt pathway and is predicted to be effective in disorders where the mTOR growth control pathway is affected.[6] mTOR is overexpressed in some vascular malformations; mTOR inhibitors exert their antiangiogenic activity in tumors by impairing production of VEGF.[18] This patient experienced new bone formation and disappearance of CSF rhinorrhea after drug treatment. Radiological examination revealed an obvious increase in bone density (Fig. 2A). The precise mechanism of new bone formation is unknown. We speculate that antiangiogenic therapy inhibited proliferation of vascular and lymphatic endothelial cells in the osteolytic lesion and led to new bone formation and disappearance of CSF rhinorrhea.

In conclusion, we present the case of a child with GSD involving the skull base who presented with CSF leakage, severe hearing loss, and facial palsy secondary to herniation of cerebellar tissue into the IAC. Antiangiogenic therapy might inhibit proliferation of vascular endothelial cells in osteolytic lesions and lead to new bone formation.

ACKNOWLEDGMENTS

We thank Dr. Nobuyuki Yotani and Dr. Nobuhito Morota at the National Center for Child Health and Development and Dr. Toshiya Kamiya at Matsuzaka General Hospital for their helpful comments. We also thank the Department of Pediatrics at Gifu University for their contribution. The present study was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan (25461587); a Health and Labour Science Research Grant for Research on Intractable Diseases from the Ministry of Health, Labour and Welfare of Japan received by M.O.; and Practical Research Project for Rare/Intractable Diseases from Japan's Agency for Medical Research and Development, AMED (15Aek0109057h0102).

REFERENCES

1. Gorham LW, Wright AW, Shultz HH, Maxon FC Jr. Disappearing bones: A rare form of massive osteolysis: Report of two cases, one with autopsy findings. Am J Med 1954;17:674–682.
2. Dellinger MT, Garg N, Olsen BR. Viewpoints on vessels and vanishing bones in Gorham-Stout disease. Bone 2014;13:47–52.
3. Halliday DR, Dahlin DC, Pugh DG, Young HH. Massive osteolysis and angiomatosis. Radiology 1964;82:637–644.
4. Iyer GV. Cerebrospinal fluid rhinorrhoea from massive osteolysis of the skull. J Neurol Neurosurg Psychiatry 1979;42:767–769.

5. Hammill AM, Wentzel M, Gupta A, Nelson S, Lucky A, Elluru R, Dasgupta R, Azizkhan RG, Adams DM. Sirolimus for the treatment of complicated vascular anomalies in children. Pediatr Blood Cancer 2011;57:1018–1024.
6. Vignot S, Faivre S, Aguirre D, Raymond E. mTOR-targeted therapy of cancer with rapamycin derivatives. Ann Oncol 2005;16:525–537.
7. Lackner H, Karastaneva A, Schwinger W, Benesch M, Sovinz P, Seidel M, Sperl D, Lanz S, Haxhija E, Reiterer F, Sorantin E, Urban CE. Sirolimus for the treatment of children with various complicated vascular anomalies. Eur J Pediatr 2015; 174:1579–1584.
8. Ozeki M, Funato M, Kanda K, Ito M, Teramoto T, Kaneko H, Fukao T, Kondo N. Clinical improvement of diffuse lymphangiomatosis with pegylated interferon alfa-2b therapy: Case report and review of the literature. Pediatr Hematol Oncol 2007;24: 513–524.
9. Girn HR, Towns G, Chumas P, Holland P, Chakrabarty A. Gorham's disease of skull base and cervical spine–confusing picture in a two year old. Acta Neurochir 2006;148:909–913.
10. Cushing SL, Ishak G, Perkins JA, Rubinstein JT. Gorham–Stout syndrome of the petrous apex causing chronic cerebrospinal fluid leak. Otol Neurotol 2010;31:789–792.
11. Morimoto N, Ogiwara H, Miyazaki O, Kitamuara M, Nishina S, Nakazawa A, Maekawa T, Morota N. Gorham–Stout syndrome affecting the temporal bone with cerebrospinal fluid leakage. Int J Pediatr Otorhinolaryngol 2013;77:1596–1600.
12. Gao K, Ma H, Cui Y, Chen X, Ma J, Dai J. Meningiomas of the cerebellopontine angle: Radiological differences in tumors with internal auditory canal involvement and their influence on surgical outcome. PLoS ONE 2015;10:e0122949.
13. Hu YF, Cheng PW, Young YH. Comparison of vestibular function between large cerebellopontine angle meningioma and schwannoma. Acta Otolaryngol 2009;129:161–165.
14. Neely JG. Reversible compression neuropathy of the eighth cranial nerve from a large jugular foramen schwannoma. Arch Otolaryngol 1979;105:555–560.
15. Radhakrishnan K, Rockson SG. Gorham's disease: An osseous disease of lymphangiogenesis? Ann N Y Acad Sci 2008;1131:203–205.
16. Hagendoorn J, Padera TP, Yock TI, Nielsen GP, di Tomaso E, Duda DG, Delaney TF, Gaissert HA, Pearce J, Rosenberg AE, Jain RK, Ebb DH. Platelet-derived growth factor receptor-beta in Gorham's disease. Nat Clin Pract Oncol 2006;3:693–697.
17. Cano B, Insa S, Cifrián C, Cortina H, Hernández M. Radiologic findings in Gorham–Stout syndrome. Radiologia 2006;48:33–36.
18. Shirazi F, Cohen C, Fried L, Arbiser JL. Mammalian target of rapamycin (mTOR) is activated in cutaneous vascular malformations *in vivo*. Lymphat Res Biol 2007;5:233–236.

Clinical Features and Prognosis of Generalized Lymphatic Anomaly, Kaposiform Lymphangiomatosis, and Gorham–Stout Disease

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Background. Complex lymphatic anomalies are intractable lymphatic disorders, including generalized lymphatic anomaly (GLA), Gorham–Stout disease (GSD), and kaposiform lymphangiomatosis (KLA). The etiology of these diseases remains unknown and diagnosis is confused by their similar clinical findings. This study aimed to clarify the differences in clinical features and prognosis among GLA, KLA, and GSD, in Japanese patients. ***Procedure.*** Clinical features, radiological and pathological findings, treatment, and prognosis of patients were obtained from a questionnaire sent to 39 Japanese hospitals. We divided the patients into three groups according to radiological findings of bone lesions and pathology. Differences in clinical findings and prognosis were analyzed. ***Results.*** Eighty-five patients were registered: 35 GLA, 9 KLA, and 41 GSD. Disease onset was more common in the first two decades of life (69 cases). In GSD, osteolytic lesions were progressive and consecutive. In GLA and KLA, 18 patients had osteolytic lesions that were multifocal and nonprogressive osteolysis. Thoracic symptoms, splenic involvement, and ascites were more frequent in GLA and KLA than in GSD. Hemorrhagic pericardial and pleural effusions were more frequent in KLA than GLA. GSD had a significantly favorable outcome compared with combined GLA and KLA ($P = 0.0005$). KLA had a significantly poorer outcome than GLA ($P = 0.0268$). ***Conclusions.*** This survey revealed the clinical features and prognosis of patients with GLA, KLA, and GSD. Early diagnosis and treatment of KLA are crucial because KLA has high mortality. Further prospective studies to risk-stratify complex lymphatic anomalies and optimize management for KLA are urgently needed. Pediatr Blood Cancer © 2016 Wiley Periodicals, Inc.

Key words: complex lymphatic anomaly; generalized lymphatic anomaly; Gorham–Stout disease; kaposiform lymphangiomatosis; lymphatic malformation; osteolysis

INTRODUCTION

Complex lymphatic anomaly is a recently proposed disease category of intractable lymphatic disorders, including generalized lymphatic anomaly (GLA) and Gorham–Stout disease (GSD).[1] The current literature is confined to case reports and several small series because of the low incidence of these diseases. The clinical features and mortality rate of the patients remain unknown. In some patients, proper diagnosis is difficult because the clinical findings are overlapping.

GLA is characterized by diffuse or multicentric proliferation of dilated lymphatic vessels resembling common lymphatic malformation (LM). The International Society for the Study of Vascular Anomalies (ISSVA) has recently suggested replacing the term "lymphangiomatosis" with GLA. This is because the suffix "oma" implies neoplastic proliferation.[2] GLA may present at birth but may also occur in children and young adults. It has a variable presentation and can affect several different sites including bone, liver, spleen, mediastinum, lungs, and soft tissues. The clinical course is directly related to the affected sites and extent of the disease.[3] Thoracic involvement may be associated with poor prognosis compared with cases with soft tissue or bone involvement.[4]

GSD is a rare disease characterized by osteolysis in bony segments, with localized proliferation of lymphatic or vascular channels in areas adjacent to the affected bone.[5] Several bones may become involved and these undergo progressive destruction and resorption. Areas commonly affected by GSD include ribs, cranium, clavicle, and cervical spine.[6] Pain and swelling in the affected area may occur. While GSD mainly involves the skeletal system, it can also involve the viscera, and clinical findings of GSD and GLA closely overlap.[7] Lala et al. have reported that patients with GLA and GSD displayed differences in the radiological findings of their bone lesions; GLA patients have lytic areas confined to the medullary cavity, whereas GSD patients have progressive osteolysis resulting in the loss of cortical bone.[6] Although these diseases are known as different conditions, affected bones from both groups of patients show abnormal lymphatic channels and appear histologically similar. Further study of GLA and GSD will help delineate the clinical,

Additional Supporting Information may be found in the online version of this article.

Abbreviations: CT, computed tomography; GLA, generalized lymphatic anomaly; GSD, Gorham–Stout disease; ISSVA, International Society for the Study of Vascular Anomalies; KLA, kaposiform lymphangiomatosis; LM, lymphatic malformation; MRI, magnetic resonance imaging

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Grant sponsor: National Center for Child Health and Development (24-19); Grant sponsor: Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan; Grant number: 25461587; Grant sponsor: Health and Labour Science Research Grant for Research on Intractable Diseases from the Ministry of Health, Labour and Welfare of Japan; Grant sponsor: Practical Research Project for Rare/Intractable Diseases from Japan's Agency for Medical Research and Development, AMED; Grant number: 15Aek0109057h0102.

Conflict of interest: Nothing to declare.

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Received 20 August 2015; Accepted 30 December 2015

DOI 10.1002/pbc.25914
Published online in Wiley Online Library (wileyonlinelibrary.com).

histological, and genetic similarities and differences between these two rare diseases.

Kaposiform lymphangiomatosis (KLA) has recently been distinguished as a novel subtype of GLA with foci of spindle endothelial cells amid a background of malformed lymphatic channels.[8] All cases of KLA involve multiple organs with a predilection for the thoracic cavity, causing pleural effusion that commonly leads to respiratory distress and dyspnea.

The etiology of these diseases is poorly understood and it is likely that the diseases represent a clinical spectrum of lymphatic pathological processes. However, recent studies about this spectrum of lymphatic diseases have suggested differences in the clinical characteristics of these complex lymphatic anomalies. The purposes of the present study were to investigate the clinical features of patients with these diseases, and to clarify the differences among the diseases and their prognoses.

METHODS

Ethical Approval, Organization, Questionnaire, and Data Collection

The Institutional Review Board of Gifu University Graduate School of Medicine approved this study. To elucidate the clinical characteristics of GLA and GSD, we conducted a nationwide, questionnaire-based survey in Japan under the auspices of the Japanese Ministry of Health, Labour and Welfare Research Program into Intractable Diseases Research Grants. The first set of questionnaires was sent to 520 Japanese hospitals with pediatric departments. The first questionnaire queried how many patients with GLA and GSD the target hospitals had between 2000 and 2013. We did not ask about KLA because it was not a known disease at the start of the study. The second set of questionnaires, which focused on clinical information such as age at onset and diagnosis, family history, perinatal history, symptoms, lesion site, radiographic and pathological findings, treatment, complications, clinical course, and outcome of patients, was sent to their attending physicians. Additionally, we requested that the authors, who had reported GLA and GSD in the literature in the past 10 years, complete the second questionnaire. To avoid reporting duplicate data, we identified overlapping patients by date of birth, sex, home village, and time of diagnosis.

Data Curation, Review of Pathological and Radiological Examinations, and Diagnosis

We categorized collected patients into GLA, KLA, and GSD according to pathological and radiological examinations. Exclusion criteria included diagnosis of other lymphatic diseases, for example, localized common LM lesions without extensive involvement, central conductive lymphatic anomaly, and lymphedema, or insufficient data. The pathological examinations were reviewed retrospectively. We defined as KLA foci of spindle endothelial cells amid a background of malformed lymphatic channels.[8] As noted previously, the key features of GSD are osteolysis and disappearing bone.[9] To diagnose GSD, the radiological findings of all patients were reviewed. Thus, only patients with evidence of cortical loss and/or progressive bone resorption were categorized as having GSD.[6] Patients with evidence of lytic bone confined to the medullary cavity or without bone lesions were diagnosed with GLA. Clinical data of these patients were carefully reviewed by two pediatric specialists (T.H. and K.K.). Pathological examinations were reviewed independently by a pediatric pathologist (K.M.). Imaging examinations were reviewed independently by a pediatric radiologist (S.N.). In cases of diagnostic discrepancy, a final decision was reached by consensus.

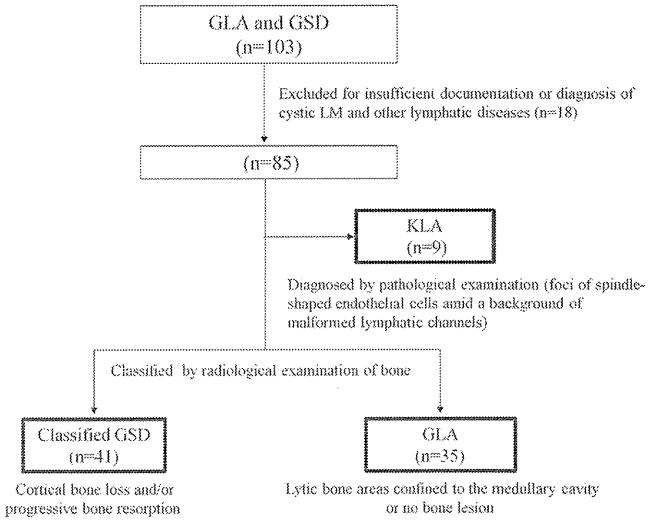

Fig. 1. Diagnostic charts of GLA, KLA, and GSD. GLA, generalized lymphatic anomaly; GSD, Gorham–Stout disease; KLA, kaposiform lymphangiomatosis.

Data Analysis

Because of the similarity between GLA and KLA, the findings including the number and distribution of bones involved in these two groups combined were initially compared with GSD, and then specific differences between GLA and KLA were sought. Prognosis as measured by overall survival was compared in the same way.

Statistical analysis was performed with GraphPad Prism version 6. Descriptive statistical methods (median and standard deviation), Wilcoxon's rank sum test for comparison of age, the number of bones involved, duration from symptom onset to diagnosis and follow-up period, and Fisher's exact test for two-group comparison, were used for the statistical analyses. Overall survival was analyzed from the date of onset by the product–sum method of Kaplan–Meier. The differences in survival times between the combined GLA and KLA group and GSD group, or GLA group and KLA group, were compared with the log-rank test. A value of $P < 0.05$ was considered statistically significant.

RESULTS

Patients and General Characteristics at Study Entry

A total of 420 responses (80.7% response rate) were received to the first set of questionnaires. A second questionnaire was then sent to 39 institutions, asking for information regarding clinical features, treatments, and outcomes for each patient. One hundred and three patients were diagnosed with GLA and GSD.

Eighteen cases were excluded because of insufficient documentation or diagnosis of other lymphatic diseases. Therefore, 85 patients were included in our study. Pathological examination was available in 71 patients (83.5%). It was not available in 14 patients because of the following reasons: examination was not performed (n = 6), pathological specimens were already disposed or for internal hospital use only (n = 4), or we could not obtain the cooperation of the primary doctor (n = 4). We categorized these patients into GLA or GSD according to pathological diagnosis in the survey center and radiological examinations. The number of patients with GSD, GLA, and KLA was 41, 35, and 9, respectively (Fig. 1). Among 44 patients with GLA and KLA, 39 (88.6%) underwent pathological examination for confirmation of diagnosis. Two patients did not undergo pathological examination because they died from rapid progression of respiratory failure. These two patients also had severe thrombocytopenia and coagulation disorder at clinical diagnosis. Another three patients without pathological examination were all infants (0, 7, and 12 months old) and their diagnosis was made by radiological examination alone.

The clinical characteristics of the patients are shown in Supplemental Table I. No family and perinatal history was evident for any of these patients. There was no significant difference in the male-to-female ratio among the three groups of patients. The median age of all patients at the time of data collection was 19.0 years (range: 1.3–70 years). The median ages of the GLA, KLA, and GSD groups were 18.0 (range: 1.3–70), 10.0 (range: 1.5–18.5), and 21.9 (range: 1.5–64.7) years, respectively. Age of onset ranged from infancy to adulthood (mean: 12.0, range: 0–63 years) but disease was more common in the first two decades of life (69/85, 81.2%), and 18 patients were aged <1 year, most of whom (12 infants) had GLA. The duration between onset and diagnosis in the three groups did not differ significantly. However, the duration in the KLA patients tended to be shorter than in other groups.

Characteristics of Affected Areas

Bone lesions, being a diagnostic criterion, were present in all patients diagnosed with GSD, but were also present in 40.9% (18 of 44) of GLA or KLA patients (Supplemental Table II). Osteolysis was the most common finding. The typical bone resorption in GSD is shown in Figures 2A, 2C, and 2D. In GLA and KLA, 16 of 44 (36.4%) patients had osteolysis, which was characterized by multiple lytic changes (Figs. 3A–3C). There were no significant radiological differences in the bone lesions between GLA and KLA. In GSD, pathological fracture was more frequent. A greater number of bones were involved in GLA or KLA than in GSD (Supplemental Table III). The spine was the most common site of osseous changes in all patients, and the spine lesions were identified more often in GLA or KLA than GSD (Fig. 3C). In GSD, 19 patients (54.3%) had bone lesions in any of the four limbs (14 femur, four humerus, nine lower leg, and two tarsal bone). With regard to skeletal axis of the osteolytic lesion, the axial skeleton (skull, spine, and ribs) was affected in 16 of the 18 patients with GLA or KLA. However, the appendicular skeleton (scapula, clavicle, pelvis, ilium, and four limbs) was only affected in six patients. An infiltrative soft tissue abnormality adjacent to the area of osseous involvement was identified more often in GSD than GLA or KLA ($P \leq 0.001$).

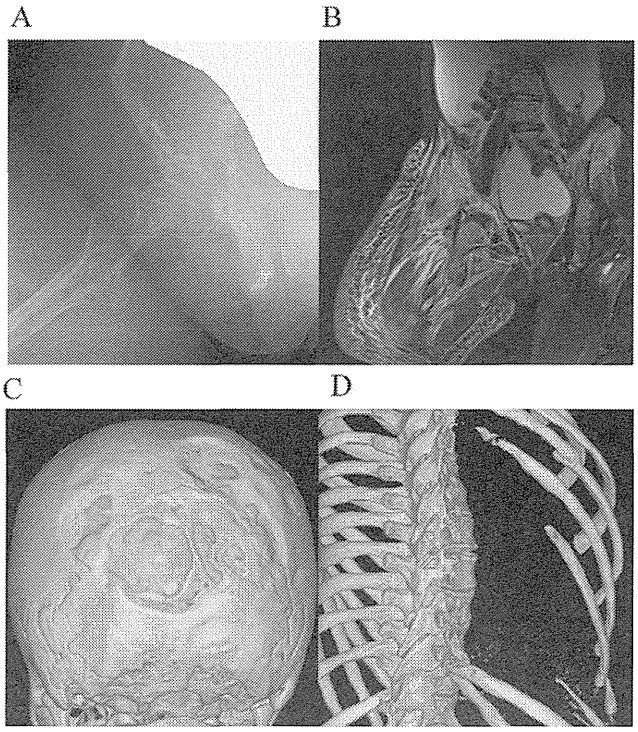

Fig. 2. Radiological findings of patients with GSD. (**A** and **B**) Thirteen-year-old female with GSD involving right lower extremity, right pelvis, spine, abdomen, and right thorax. (**A**) Plain radiograph of the right pelvis shows osteolysis at the proximal femoral shaft as well as the pelvic bone. (**B**) Coronal fat suppressed in T2-weighted image of the pelvis demonstrates extensive high signal intensity in the soft tissue surrounding the femur as well as subcutaneous fat tissue. Involved right pelvis shows high signal intensity secondary to osteolysis. Also visible is a large amount of ascites. (**C**) Nine-year-old male with GSD involving the occipital and temporal bones. Posterior view of 3D CT imaging shows almost complete resorption of the occipital bone. (**D**) Thirteen-year-old female with GSD involving the ribs. Right oblique posterior view of 3D CT shows multiple osteolysis and fracture of the posterior portion of the right 5–10 ribs. CT, computed tomography; GSD, Gorham–Stout disease (GSD).

Thoracic lesions were the second most common, with symptoms of cough, chest pain, dyspnea, and wheezing. Some patients experienced wheezing and were misdiagnosed with asthma prior to recognition of the lymphatic disorder. Pleural effusion, mediastinal mass, and cardiac effusion were significantly more frequent in GLA and KLA than GSD ($P = 0.002$, 0.002, and 0.001, respectively). The frequency of pleural and cardiac effusion in KLA was similar to that in GLA, but mediastinal masses and hemorrhagic pericardial and pleural effusions were more frequent in KLA than GLA ($P = 0.017$ and <0.001, respectively). The other findings were heart failure, cardiac tamponade, and pulmonary infiltration and hemorrhage. In abdominal lesions, splenic involvement and ascites were more frequent in GLA and KLA than GSD ($P = 0.044$ and 0.001, respectively). However, KLA patients did not have ascites. Seven patients had cystic LM of the mesentery and retroperitoneum, in combination with ascites or invasion of the surrounding soft tissue. Skin lesions were present in 36 of 85 (42.3%) cases. Three patients

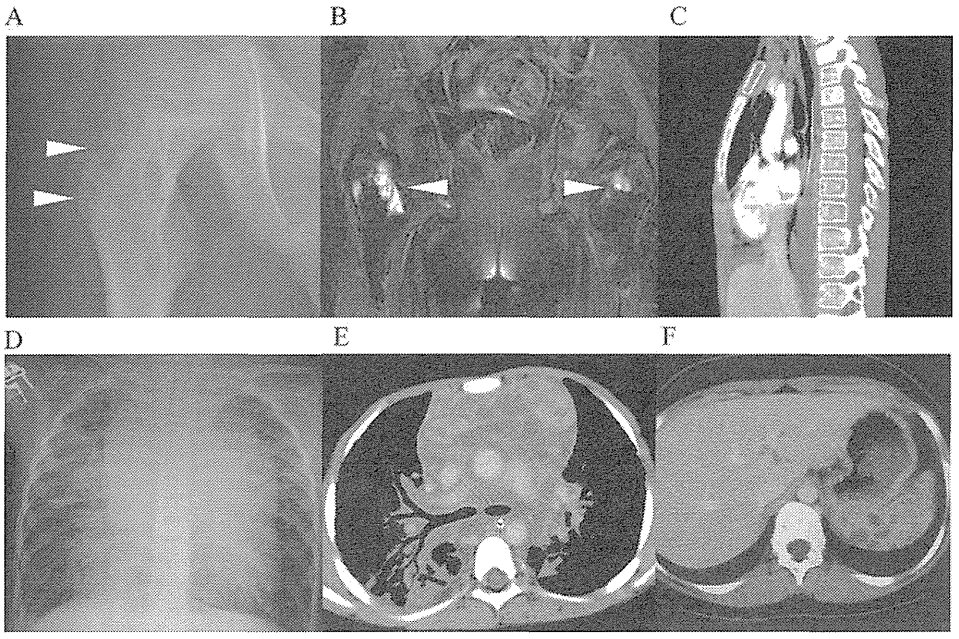

Fig. 3. Radiological findings of patients with GLA and KLA. (**A–C** and **F**) Twelve-year-old male with GLA involving bone and spleen. (**A**) Plain radiography of femoral head shows a small lytic lesion (white arrowheads). (**B**) Coronal fat suppressed, T2-weighted MRI of femoral head shows a small lytic lesion (white arrowheads). (**C**) Contrast-enhanced CT of the chest with sagittal reconstruction shows dissemination of osteolysis in the vertebrae. (**D** and **E**) Eight-year-old male with KLA involving the thoracic region. (**D**) Chest radiography shows pronounced effusion, mediastinal enlargement, and peribronchovascular infiltration extending from the hilar to the peripheral area. (**E**) Chest contrast-enhanced CT demonstrates diffuse thickening of the interlobular septa, pleural effusion in the right lung, and retroperitoneal soft tissue mass (red arrows). (**F**) An axial projection shows multiple cystic lesions in the spleen. CT, computed tomography; GLA, generalized lymphatic anomaly; KLA, kaposiform lymphangiomatosis; MRI, magnetic resonance imaging.

had subcutaneous macrocytic LMs at birth. Neurological disorders were uncommon (10/85 [11.8%]) and were associated with osteolytic lesions of the skull.

Imaging and Histopathological Examination

Bone lesions were present in 59 of 85 cases and imaging examinations of bone lesions included plain radiography (n = 59), computed tomography ([CT]; n = 59), magnetic resonance imaging ([MRI]; n = 57), and bone scintigraphy (n = 3). CT showed more definite location and number of osteolytic lesions than did plain radiography. MRI demonstrated altered signaling in bone marrow, which was hypointense on T1-weighted imaging. An infiltrative soft tissue abnormality adjacent to the area of osseous involvement was characterized by high signal intensity in T2-weighted imaging, which indicated lymphatic infiltration or accumulation of lymphatic fluid (Fig. 2B). Bone scintigraphy demonstrated increased uptake of radiotracer in the pathological fracture of one case, while there was no increased uptake in the osteolytic lesion. In thoracic lesions of typical patients with GLA or KLA, bilateral interstitial infiltrates in the lung and pericardial or pleural effusions were evident on chest radiography (Fig. 3D). Thoracic CT revealed diffuse smooth thickening of interlobular septa and bronchovascular bundles, with extensive involvement of mediastinal connective tissue and perihilar regions. The thickening and soft tissue mass of the pleura, posterior mediastinum, and anterior ribs were characteristic findings, especially in KLA (Fig. 3E). Thirty-three patients had splenic involvement, identified by CT (n = 33), MRI (n = 29), and ultrasound (n = 20). These lesions were multiple cystic lesions in most cases (Fig. 3F). On Doppler ultrasound, the lesions did not demonstrate increased vascular flow. Histopathology demonstrated anastomosing endothelium-lined spaces consistent with lymphatic vessels stained with D2-40 (Figs. 4A–4F). Although KLA was diagnosable by characteristic findings at pathological examination, there were no significant histopathological differences between GLA and GSD. Bone biopsy specimens showed dilated lymphatic lumens containing lymphatic fluid in lytic lesions; however, some specimens were not in sufficiently good condition to reconstruct their architecture for diagnosis.

Treatments

Treatment included medication, surgery, radiotherapy, and nutritional therapy. In patients with bone lesions, medical treatment included interferon-α, propranolol, bisphosphonates, and corticosteroids. When these medications were not effective, patients needed to undergo surgery or symptomatic treatment. Surgical interventions included resection of the lesion and orthopedic operations (fracture reduction or reconstruction). In some patients, surgical reconstruction undertaken to control the disease was unsuccessful because of rapid osteolysis and resorption of bone graft material. The common surgical procedures for pleural effusion were pleurocentesis, pleurodesis, and ligation of the thoracic duct. Medical therapy for thoracic lesions included corticosteroids, propranolol, interferon-α, octreotide, and mammalian target of rapamycin (mTOR) inhibitor. Sixteen patients

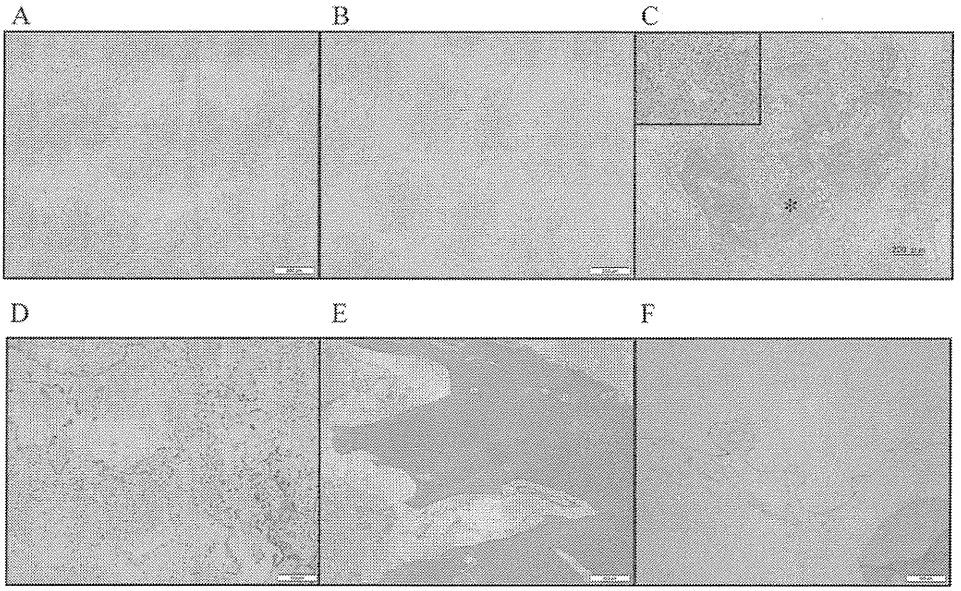

Fig. 4. Pathological findings in patients with GLA, KLA, and GSD. (**A** and **B**) Specimen of subcutaneous lesion from GLA patient. (**A**) This specimen shows proliferation of thin-walled, anastomosing lymphatic vessels lined by a single layer of endothelial cells without foci of spindle endothelial cells (bar 200 μm, hematoxylin and eosin [H&E]). (**B**) Endothelial cells were identified as lymphatic using D2-40 (bar 100 μm). (**C** and **D**) Specimen of thoracic lesion from KLA patient. (**C**) Specimen shows proliferation of thin-walled, anastomosing lymphatic vessels lined by a single layer of endothelial cells with a focus of spindle cells (*) (bar 200 μm, H&E). Spindle cells can be seen in the insert image. (**D**) Endothelial cells were identified as lymphatics using D2-40 (bar 100 μm). (**E** and **F**) Specimen of femur biopsy affected by GSD. (**E**) Typical bone structures were resorbed and replaced by thin-walled endothelium-lined capillaries of vascular or lymphatic origin (bar 200 μm, H&E). (**F**) D2-40 immunostaining delineates the endothelium of lymphatic channels (bar 100 μm, H&E). GLA, generalized lymphatic anomaly; GSD, Gorham–Stout disease; KLA, kaposiform lymphangiomatosis.

(six with bone lesions of GSD, and 10 with thoracic lesions) underwent radiotherapy. The total doses applied and fractionation regimens varied widely among patients and lesions. Nutritional therapy (fat-restricted diet and low-fat medium chain triglyceride diet) had no effects in almost all patients.

Follow-up Period and Mortality

Follow-up was available for all patients. The mean follow-up period was 7.0 (range, 0.1–32; median, 4) years. Overall or in aggregate, mortality rate was 20% (17/85) and the cause of death was thoracic symptoms (Fig. 5A). All 29 patients (nine adults and 20 children) who lacked thoracic lesions survived. Of the 69 pediatric patients, 50 had thoracic lesions and 13 died (26%). The GSD group had a significantly more favorable outcome than the combined GLA and KLA group had ($P = 0.0005$) (Fig. 5B). In contrast, the KLA patients had a significantly poorer outcome than the GLA patients had ($P = 0.0268$) (Fig. 5C).

DISCUSSION

In this nationwide retrospective observational study, we collected data on follow-up of patients with GLA, KLA, or GSD from Japanese hospitals. This study demonstrated the frequency and features of principal symptoms, diagnostic signs, and prognosis. Our data contribute to understanding the clinical features and prognosis of these diseases.

Osteolytic lesions are important for differential diagnosis. They can be caused by several different conditions including infection, inflammation, cancer, and endocrine disorders. In our study, some cases were misdiagnosed as Langerhans cell histiocytosis and osteomyelitis before definitive diagnosis. There is no specific test or procedure to diagnose GSD definitively;[3] thus, diagnosis is based upon identification of characteristic symptoms, detailed patient history, radiological examination, and histopathological findings. Especially, radiological findings of osteolysis seem important to distinguish among GLA, KLA, and GSD. Patients with GLA and KLA displayed lytic areas confined to the medullary cavity, whereas patients with GSD showed progressive osteolysis resulting in the loss of cortical bone. GLA and KLA typically involved more bones than GSD did. The bone lesions of GSD were often progressive and sequentially infiltrative; in contrast, those of GLA and KLA were nonprogressive. However, our study also showed that there were some overlapping features and no obvious difference in bone histopathology.

KLA was recently distinguished as a novel subtype of GLA.[8,10] Although dilated malformed lymphatic channels lined by a single layer of endothelial cells are common to both GLA and KLA, the latter also has foci of patternless clusters of intra- or perilymphatic spindled cells associated with platelet microthrombi, extravasated red blood cells, hemosiderin, and some degree of fibrosis. Patients with KLA also have coagulation disorders, and hemorrhagic pericardial and pleural effusions.[8] In our study, nine patients were diagnosed pathologically with KLA from among those who were diagnosed with GLA. The multiple overlapping clinical characteristics and radiological findings of GLA have led to a hypothesis that KLA may arise from GLA. There have been no cases of

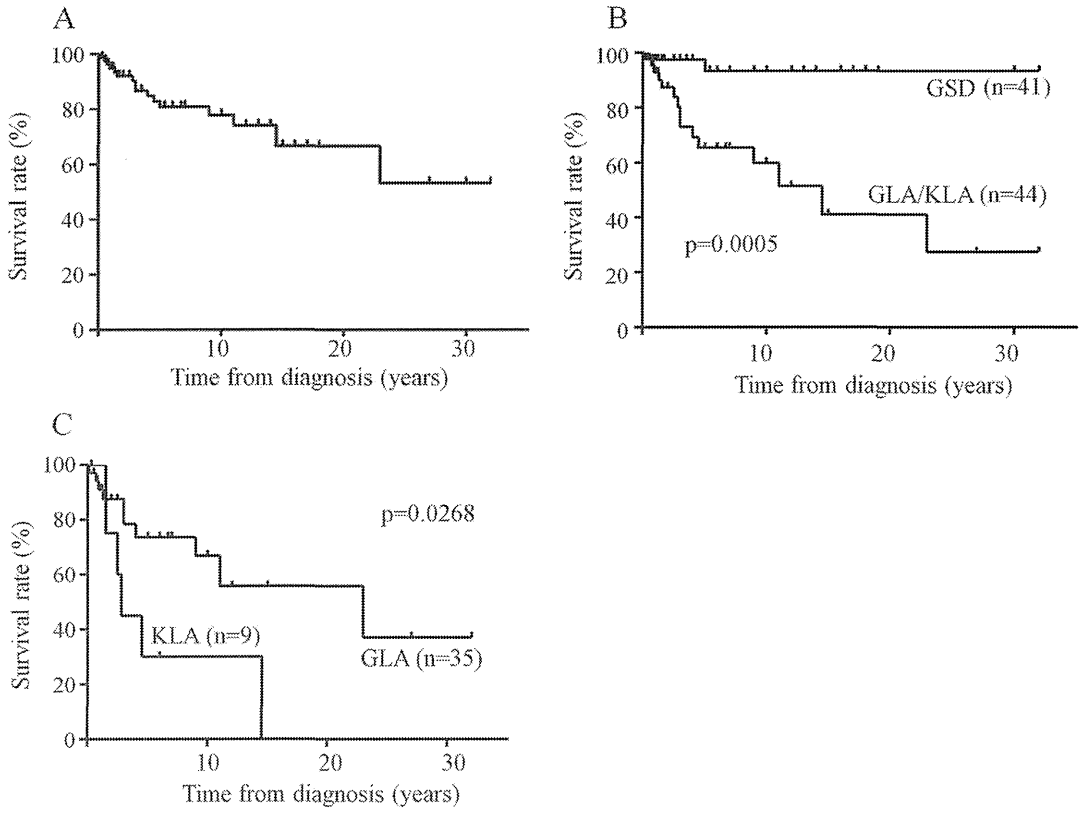

Fig. 5. Kaplan–Meier curve showing time from onset. (**A**) Overall survival of all cases (n = 85). (**B**) Overall survival in GSD group (n = 41) compared with combined GLA and KLA group (n = 44). The GSD group had a significantly more favorable outcome than the combined GLA and KLA group had ($P = 0.0005$) (**C**). Overall survival in GLA group (n = 35) compared with KLA group (n = 9). KLA group had a significantly poorer outcome than the GLA group had ($P = 0.0268$). GLA, generalized lymphatic anomaly; GSD, Gorham–Stout disease; KLA, kaposiform lymphangiomatosis.

GLA that have evolved into KLA over several months or years. However, patients with KLA had unfavorable prognosis and serious symptoms (hemorrhagic pericardial and pleural effusions). We might have to distinguish KLA from GLA or other LMs.

In patients with multifocal lesions, therapeutic options are palliative, and therapy is often aimed at reducing symptoms associated with bone lesions and chylous effusions.[11] Management of chylothorax is often the primary concern. Thoracentesis and pleural drainage are used to treat chylothorax to relieve respiratory distress. In the case of failure with such conservative treatment, thoracic duct ligation has been used to treat pleural effusion in isolated cases.[4] Radiotherapy has been used in cases in which surgery is not possible, or in combination with surgery. Several case reports have described the successful use of radiotherapy, achieving pain relief and arresting the spread of osteolysis. Positive results have been achieved with a total dose of 30–45 Gy.[11]

Patients with osteolysis have been treated with drugs that inhibit bone resorption, including bisphosphonates such as pamidronic or zoledronic acid.[12] Patients with bone or thoracic lesions have been treated with interferon-α-2b, which inhibits the formation of lymphatic vessels.[13] Other pharmaceuticals include vascular endothelial growth factor-A antibody, bevacizumab,[14] propranolol,[15] steroids, vitamin D, and calcitonin. These treatments, used alone or in combination, improve symptoms in some cases, but their effectiveness is inconsistent. A recent study suggested that the antilymphangiogenic properties of rapamycin (sirolimus; an mTOR inhibitor) and its derivatives might have therapeutic value for the prevention and treatment of malignancies.[16] In our study, there were nine patients (eight with thoracic and seven with bone lesions) who were treated with an mTOR inhibitor. Further investigation is needed to determine which treatments are effective and safe in these patients.

It is necessary to address the limitations and bias of the present study. This was a retrospective survey that was limited to general hospitals with pediatric departments and physicians who reported cases. There was also responder bias in that providers managing the most severe cases may have been more likely to report them. When we started this survey in 2013, a new classification of ISSVA had not been announced and there were no standard diagnostic criteria for complex lymphatic anomalies. Therefore, there was some possible involvement of other similar diseases. Multifocal lymphangioendotheliomatosis with thrombocytopenia, multifocal kaposiform hemangioendothelioma, and central conducting lymphatic anomalies are rare diseases, but could be misdiagnosed as GLA.[17,18] Patients diagnosed with GLA might have KLA because of a lack of characteristic findings at pathological examination. In

addition to the above limitations, it is probable that classification of vascular anomalies will be rationally modified according to advances in genetic and clinicopathological research in the future. Despite the above-mentioned limitations, this study is noteworthy because it was a large detailed survey of GLA, KLA, and GSD. We believe that sufficient data were collected to characterize the frequency of principal symptoms, diagnostic signs, and prognosis.

In conclusion, we performed a nationwide, questionnaire-based survey of complex lymphatic anomalies, GLA, GSD, and KLA, to characterize their prevalence, clinical features, radiological and pathological findings, treatment, and prognosis. This study revealed the clinical presentation and severe course of the patients, and limited current therapeutic options. Further study into the pathogenesis and a prospective study are needed for better therapy with improved outcome, especially for KLA.

ACKNOWLEDGMENTS

This study was supported by a grant from the National Center for Child Health and Development (24-19); Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan (25461587); a Health and Labour Science Research Grant for Research on Intractable Diseases from the Ministry of Health, Labour and Welfare of Japan received by M.O.; and Practical Research Project for Rare/Intractable Diseases from Japan's Agency for Medical Research and Development, AMED (15Aek0109057h0102). We thank the cooperative institutions in Japan and Dr. Tomohiro Hori, Dr. Kaori Kanda, and Dr. Norio Kawamoto, Gifu University, for helpful comments and data review. We also thank the Department of Pediatrics at Gifu University for its contribution.

REFERENCES

1. Trenor CC 3rd, Chaudry G. Complex lymphatic anomalies. Semin Pediatr Surg 2014;23:186–190.
2. International society for the study of vascular anomalies: ISSVA classification for vascular anomalies (Approved at the 2-th ISSVA Workshop, Melbourne, April 2014). http://issva.org. Accessed April 2014.
3. Blei F. Lymphangiomatosis: Clinical overview. Lymphat Res Biol 2011;9:185–190.
4. Alvarez OA, Kjellin I, Zuppan CW. Thoracic lymphangiomatosis in a child. J Pediatr Hematol Oncol 2004;26:136–141.
5. Radhakrishnan K, Rockson SG. Gorham's disease: An osseous disease of lymphangiogenesis? Ann N Y Acad Sci 2008;1131:203–205.
6. Lala S, Mulliken JB, Alomari AI, Fishman SJ, Kozakewich HP, Chaudry G, Gorham–Stout disease and generalized lymphatic anomaly – Clinical, radiologic, and histologic differentiation. Skeletal Radiol 2013;42:917–924.
7. Michael TD, Nupur G, Bjorn RO. Viewpoints on vessels and vanishing bones in Gorham–Stout disease. Bone 2014;63:47–52.
8. Croteau SE, Kozakewich HP, Perez-Atayde AR, Fishman SJ, Alomari AI, Chaudry G4, Mulliken JB, Trenor CC 3rd. Kaposiform lymphangiomatosis: A distinct aggressive lymphatic anomaly. J Pediatr 2014;164:383–388.
9. Gorham LW, Stout AP. Massive osteolysis (acute spontaneous absorption of bone, phantom bone, disappearing bone); its relation to hemangiomatosis. J Bone Joint Surg Am 1955;37-A:985–1004.
10. Dasgupta R, Fishman SJ. ISSVA classification. Semin Pediatr Surg 2014;23:158–161.
11. Hagendoorn J, Yock TI, Borel Rinkes IH, Padera TP, Ebb DH. Novel molecular pathways in Gorham disease: Implications for treatment. Pediatr Blood Cancer 2014;61:401–406.
12. Heyd R, Micke O, Surholt C, Berger B, Martini C, Füller J, Schimpke T, Seegenschmiedt MH. German Cooperative Group on Radiotherapy for Benign Diseases (GCG-BD). Radiation therapy for Gorham–Stout syndrome: Results of a national patterns-of-care study and literature review. Int J Radiat Oncol Biol Phys 2011;81:179–185.
13. Kuriyama DK, McElligott SC, Glaser DW, Thompson KS. Treatment of Gorham–Stout disease with zoledronic acid and interferon-alpha: A case report and literature review. J Pediatr Hematol Oncol 2010;32:579–584.
14. Ozeki M, Funato M, Kanda K, Ito M, Teramoto T, Kaneko H, Fukao T, Kondo N. Clinical improvement of diffuse lymphangiomatosis with pegylated interferon alfa-2b therapy: Case report and review of the literature. Pediatr Hematol Oncol 2007;24:513–524.
15. Grunewald TG, Damke L, Maschan M, Petrova U, Surianinova O, Esipenko A, Konovalov D, Behrends U, Schiessl J, Wörtler K, Burdach S, von Luettichau I. First report of effective and feasible treatment of multifocal lymphangiomatosis (Gorham–Stout) with bevacizumab in a child. Ann Oncol 2010;21:1733–1734.
16. Ozeki M, Fukao T, Kondo N. Propranolol for intractable diffuse lymphangiomatosis. N Engl J Med 2011;364:1380–1382.
17. Reinglas J, Ramphal R, Bromwich M. The successful management of diffuse lymphangiomatosis using sirolimus: A case report. Laryngoscope 2011;121:1851–1854.
18. Nakaya T, Morita K, Kurata A, Ushiku T, Igarashi T, Kuroda M, Fukayama M. Multifocal kaposiform hemangioendothelioma in multiple visceral organs: An autopsy of 9-day-old female baby. Hum Pathol 2014;45:1773–1777.
19. Kline RM, Buck LM. Bevacizumab treatment in multifocal lymphangioendotheliomatosis with thrombocytopenia. Pediatr Blood Cancer 2009;52:534–536.